J. Dairy Sci. 109:1082–1095
https://doi.org/10.3168/jds.2025-27260

Study of changes in unripened soft rennet-curd cheeses caused by the addition of dried nettle leaves: Physicochemical properties, microbial quality, polyphenol content, and sensory characteristics

A. Pluta-Kubica,[1]* D. Najgebauer-Lejko,[1] P. Pająk,[2] J. Sobolewska-Zielińska,[2] Ł. Skoczylas,[3] A. C. K. Aththota Gamage,[1] A. Kratochvílová,[4] R. N. Salek,[4] I. Duda,[1] and J. Domagała[1]
[1]Department of Animal Product Processing, Faculty of Food Technology, University of Agriculture in Krakow, 30-149 Krakow, Poland
[2]Department of Food Analysis and Quality Assessment, Faculty of Food Technology, University of Agriculture in Krakow, 30-149 Krakow, Poland
[3]Department of Plant Products Technology and Nutrition Hygiene, Faculty of Food Technology, University of Agriculture in Krakow, 30-149 Krakow, Poland
[4]Department of Food Technology, Faculty of Technology, Tomas Bata University in Zlin, 760 01 Zlin, Czech Republic

ABSTRACT

The objectives of the study were to investigate changes in the number of selected groups of microorganisms and the level of antioxidant activity during storage of model unripened cheeses with the addition of dried nettle leaves, to compare their sensory characteristics, as well as to determine the antioxidant properties of the obtained whey. Three groups of model unripened rennet-curd cheeses were produced: I—a control sample without a plant additive, II and III—samples with the addition of dried nettle leaves in the amount of 0.165% and 0.330% (g/100 g of milk), respectively. Advantageously, no effects of nettle addition on total microbial and *Lactococcus* counts were determined. Moreover, the application of this herb slowed down the yeast and mold growth in cheese, increased the antioxidant activity, and elevated the contents of total polyphenols, caffeic acid, *p*-coumaric acid, rutin, and ferulic acid. Additionally, the antioxidant properties of the obtained whey were also higher in comparison to control. Sensory analysis revealed that the addition of a lower amount (0.165%) of dried nettle leaves appeared to be the most favorable option, balancing improved nutritional and antioxidant value with acceptable sensory characteristics and acceptability. The study demonstrated that the application of dried nettle leaves as an addition to cheese offers many benefits.

Key words: cheese, nettle, microbiology, polyphenols, sensory analysis

INTRODUCTION

Cheeses are the most diverse group of dairy products. They include substantial amounts of essential nutrients, such as AA, peptides, proteins, fatty acids, fat, minerals, and vitamins. Key determinants of cheese quality include the chemical and microbiological composition of milk, the applied cheesemaking technology, ripening parameters, and the environmental conditions within the production facility (Ioannidou et al., 2022). In addition, the huge diversification of the product range in the cheese segment is also caused by the use of vegetables, fruits, herbs, or spices. Such additives not only increase the range of flavors of cheeses, but can also affect their microbiological quality, nutritional value, or health-promoting properties. Many herbs, including nettle (*Urtica dioica* L.), have antimicrobial and antioxidant properties (Hamedi et al., 2022).

The addition of natural flavorings in cheesemaking is common throughout the world. These flavoring materials include, but are not limited to, red pepper, dill, chives, sage, parsley, onion, garlic, thyme, and black cumin. Dried or fresh herbs and spices are usually added after the coagulation when the coagulum has been cut to form curds and the whey drained (Tarakci and Temiz, 2009). However, the risk of microbiological contamination of added flavorings should be taken into account. During the production of a few types of cheese (e.g., Emmental, Parmigiano Reggiano, mozzarella, and processed cheese), the curds or cheese melt are cooked at a high temperature, and therefore, storing other cheese types with added herbs or spices may be associated with the risk of developing undesirable microflora.

Nettle is a wild plant common not only in Poland or in Europe, but also in Asia and North and South America. Nettle leaves are abundant with diverse bioactive compounds. They contain many minerals (Fe, K,

Received July 17, 2025.
Accepted November 2, 2025.
*Corresponding author: agnieszka.pluta-kubica@urk.edu.pl

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

Ca, Mg), vitamins (such as A, B, and C), phenolic acids, flavonoids, carotenoids, and chlorophylls. Nettle has anticancer, antiallergic, antioxidant, anti-inflammatory, hypoglycemic, diuretic, and antibacterial effects (Fiol et al., 2016; Repajić et al., 2021; Hamedi et al., 2022). It is mainly consumed in the form of an infusion, but it is also added during the manufacture of ripened cheese varieties, to season fresh acid-curd cheese (tvorog, also called quark, quarg, or tvarog) with sour cream dressing, and to prepare homemade soups and salads. Nettle, as a plant with antimicrobial properties (Bhusal et al., 2022), can probably extend the shelf-life of unripened cheeses (e.g., soft rennet-curd ones) in terms of their microbial quality. This type of cheese is usually produced on an artisanal basis, on agritourism farms, not on a large scale. Unripened cheeses are ready for consumption shortly after manufacture (CODEX STAN 221-2001, 2010; Codex Alimentarius Commission, 2010).

Scientific research on nettle focuses mainly on the analysis of the effect of extracts of various parts of this plant, both in vitro and in vivo (Upton, 2013; Hamedi et al., 2022). The use of nettle in cheeses has so far been limited in the literature to the investigation of its influence on color and texture of Kashar cheese during ripening (Aydın and Tarakçı, 2021) and the application of fresh nettle leaves as a coagulant of milk in cheese production (Fiol et al., 2016). However, there is no research aimed at determining the effect of adding dried nettle leaves on the microbial counts in cheeses, its antioxidant properties or sensory characteristics, or on the characteristics of obtained whey. Therefore, the use of dried nettle leaves for such purposes in a model soft rennet-curd unripened cheese is innovative. The use of an extract is not justified in this case because most of the bioactive components would be separated during the production of cheese with whey. Using naturally occurring and environmentally common herbs to extend the shelf-life of cheeses can not only reduce food losses, but also develop functional products with health-promoting properties, including those using whey.

Therefore, taking into account well-known antimicrobial and health-promoting properties of nettle, the aims of this study were to investigate changes in the number of selected groups of microorganisms and the level of antioxidant activity during storage of model unripened soft rennet-curd cheeses with the addition of dried nettle leaves, to compare their sensory characteristics, as well as to determine the antioxidant properties of the obtained whey. We hypothesized that it is possible to develop a soft unripened rennet-curd cheese with a particular level of dried nettle leaves added that would slow down the growth of yeast and molds, increase the antioxidant activity of the cheese, while maintaining acceptable sensory characteristics of the product.

MATERIALS AND METHODS

Materials

Model soft rennet cow milk cheeses were produced in laboratory conditions according to a previously described method (Pluta-Kubica et al., 2020). Three groups of cheeses were produced: I—a control sample without a plant additive, II and III—samples with the addition of dried nettle leaves in the amount of 0.165% and 0.330% (g/100 g of milk), respectively. Pasteurized homogenized milk with 2% fat content (OSM w Piątnicy, Piątnica, Poland), and dried nettle leaves with an organic certificate (Dary Natury, Grodzisk, Poland) were purchased in a local store. Milk containing 2% of fat was chosen to obtain medium fat unripened cheese in agreement with Codex Alimentarius (CODEX STAN 221-2001, Codex Alimentarius Commission, 2010). Preliminary studies (data not shown) revealed that the presence of stems and whole leaves in cheeses is negatively perceived organoleptically, and the addition of 0.165% is organoleptically acceptable, but can be increased. In addition, it was found that due to the natural contamination of dried nettle with yeasts and molds, it should be added to milk before pasteurization. Therefore, before adding to the milk, the dried nettle leaves were destemmed, chopped, and sifted for elimination of powder and particles with a diameter ≤2 mm. Adding dried leaf particles with a diameter >2 mm to the milk ensured that they remained in the cheese matrix created during coagulation and only part of the bioactive components were extracted with the whey. All 3 groups of cheeses were produced on the same day, starting with vat pasteurization of milk (sample I) and milk with nettle (samples II and III) at 72°C for 15 s. The following steps were cooling to 32°C, addition of anhydrous calcium chloride (0.2 g/kg of milk, Eurochem BGD, Tarnów, Poland) and mesophilic mixed strain starter culture CHN-19 (containing *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis*, *Leuconostoc mesenteroides* ssp. *cremoris*, and *Lactococcus lactis* ssp. *diacetylactis*, amount: 75 U per 500 L of milk, Chr. Hansen, Hørsholm, Denmark), fermentation (32°C, 1 h), addition of microbial rennet with an activity of 2,200 international milk clotting units/g (Fromase 2200TL, DSM, Heerlen, Denmark; supplied by GAP food additives, Łopuszna, Poland), coagulation (32°C, 2 h), cutting using a curd knife with a long flat blade (to obtain cheese grain measuring approximately 2 cm × 2 cm × 2 cm), stirring with a spoon (32°C, 0.5 h), molding (perforated cylindrical molds, 8 cm in diameter, 8 cm in height), draining in molds (28°C, 1 h; with inversion; 20°C, 18 h), brining (16% NaCl, 16°C–18°C, pH 5.1–5.2, 20 min), and dripping (6°C, 2 h). The pH was electrometrically determined in milk and cheese during production, using a pH meter (direct measurement, electrode ERH-12-6, CP-411, Elmetron, Zabrze, Poland). The milk before pasteurization was characterized by a pH of 6.7 ± 0.0 (± SD). Both after cooling and fermentation all milk samples had equal pH of 6.5 ± 0.0. Differences in this feature were determined after coagulation of milk (I: 6.5 ± 0.0, II: 6.4 ± 0.0, and III: 6.4 ± 0.0), after cheese draining at 28°C for 1 h (I: 6.2 ± 0.0, II: 5.9 ± 0.0, and III: 6.0 ± 0.0), after draining at 20°C for 18 h (I: 4.7 ± 0.0, II: 4.7 ± 0.0, and III: 4.8 ± 0.0), after brining (I: 4.7 ± 0.0, II: 4.7 ± 0.0, and III: 4.8 ± 0.0), and after dripping (I: 4.7 ± 0.0, II: 4.7 ± 0.0, and III: 4.8 ± 0.0).

Regarding all cheesemaking trials, cheeses I, II, and III were produced on the same day from the milk of the same batch. For the microbial and chemical analyses, each group consisted of 6 cheeses and was prepared from 4 L of milk. The cheeses were divided into 5 subgroups, which corresponded to the storage period: 0, 1, 2, 3, and 4 wk at a temperature of 6 ± 1°C. The sixth sample of each group was analyzed for polyphenol profile. Two cheesemaking trials were conducted on separate days. Regarding sensory analysis and consumer testing, each group consisted of 9 or 6 cheeses and was prepared from 6 or 4 L of milk, respectively. There were 3 cheesemaking trials to produce the samples intended for sensory analysis and one for consumer testing. Each trial was conducted on separate days.

The cheese samples weighed on average: I: 135 ± 6, II: 119 ± 2, and III: 114 ± 2 g. All samples were individually wrapped in linear low-density polyethylene film before storage. Just before the microbial and chemical analyses, the selected cheese sample was ground in a disinfected mortar and packed into a sterile test tube.

Physicochemical Properties of Cheese and Whey

The moisture and fat contents were determined according to ISO 5534:2004 (ISO, 2004) and ISO 3433:2008 (ISO, 2008a), respectively. The water activity was measured on the basis of ISO 18787:2017 (ISO, 2017b) using the LabMaster-aw water activity meter (Novasina AG, Lachen, Switzerland). The pH was electrometrically determined using a pH meter (direct measurement, electrode ERH-12-6, CP-411, Elmetron, Zabrze, Poland). All aforementioned analyses were performed directly after cheese production and following 1, 2, 3, and 4 wk of storage.

The protein, NaCl, and ash contents were analyzed according to AOAC International (2007) in cheese samples before storage. The protein, fat, and DM contents of whey were determined using a Milkoscan FT120 (FOSS, Denmark) according to PN-ISO 9622:2015-09 (2015).

Microbiological Quality of Cheese

Test samples, initial suspension, and decimal dilutions were prepared according to standard methods (PN-EN ISO 6887-5:2010, 2010). First, 10 g of each previously grated cheese sample was weighed into a stomacher bag with a full page filter (sterile, 400 mL, 190 mm × 300 mm × 0.07 mm, LABSOLUTE, Th. Geyer GmbH & Co. KG, Renningen, Germany) and homogenized with 90 mL of buffered peptone water (BioMaxima, Lublin, Poland) for 3 min using the stomacher device (Star Blender LB400, VWR, Radnor, PA). Next, appropriate decimal dilutions were prepared in buffered peptone water. Total viable count (**TVC**; PN-EN ISO 4833-1:2013-12, 2013), *Lactococcus* count (Ong and Shah, 2009), as well as yeast and mold count (PN-ISO 6611:2007, 2007) were determined directly after cheese production and following 1, 2, 3, and 4 wk of storage. Culture media such as atandard methods with powdered milk lactic acid bacteria (**LAB**)-agar, M-17 according to Terzaghi LAB-AGAR, and chloramphenicol LAB-agar, respectively, were purchased from BioMaxima.

Antioxidant Activity and Total Content of Polyphenols in Cheese and Whey

Radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (**DPPH**) of whey, fresh, and stored cheese samples was determined according to a previously described procedure (Najgebauer-Lejko et al., 2022). Briefly, 0.10 g of whey or cheese ground in a ceramic mortar was vortexed with 3.9 mL of DPPH methanolic solution (0.028 g/L) and incubated for 2 h in a dark place at ambient temperature. Subsequently, samples were centrifuged at 1,400 × g for 10 min at 20°C (MPW-352 Centrifuge, MPW Med. Instruments, Warszawa, Poland). Afterward, the absorbance of clear supernatant was read at 515 nm against a blank sample (0.10 g of methanol) using a Helios Gamma spectrophotometer (Thermo Electron Corporation, Cambridge, England). The amount of sample required to decrease the DPPH radical concentration by 50% (**EC_{50}**) was calculated. Results were expressed as antiradical power (**ARP**), the reciprocal of EC_{50}, in micromoles of Trolox equivalents (**TE**) per 1 g of cheese sample.

Preparation of extracts and subsequent total polyphenol content determination using the Folin–Ciocalteu method were performed as previously described (Najgebauer-Lejko et al., 2022). Whey and fresh and stored cheese samples were subjected to this analysis.

Polyphenolic Profile in Nettle Leaves, Whey, and Cheese

Samples for polyphenol content determination following the HPLC method were prepared according to the procedure described by Klimczak et al. (2007), with some modifications. The samples of dried nettle leaves, whey, and fresh cheese were hydrolyzed using 2 M NaOH in a dark place for 4 h (at room temperature). Then, the mixture was adjusted to pH from 2.1 to 2.6 with 2 *M* HCl (controlled using a pH meter; Metrohm, Herisau, Switzerland) and transferred quantitatively to a measuring flask with 1% L-ascorbic acid dissolved in methanol. Prior to chromatographic analysis, the samples were centrifuged (MPW-260R, Warsaw, Poland) at 30,065 × *g* (20 min, 4°C) and filtered through a Labfil polytetrafluoroethylene hydrophilic syringe filter (ALWSCI Corporation, China) with pore diameter of 0.22 µm. The samples were stored at 4°C until injection onto the column.

The chromatographic analysis was carried out in the HPLC Dionex UltiMate 3000 system with DAD detector (Thermo Scientific, Germering, Germany), equipped with a Cosmosil 5C18-MS-II column (250 × 4.6 mm i.d., 5 µm; Nacalai Tesque Inc., Kyoto, Japan). Two eluents were used as the mobile phase: A—2% (vol/vol), an aqueous solution of acetic acid, and B—100% methanol. The flow rate of the mobile phase was 1 mL/min throughout the analysis, which lasted 50 min and was performed in the following system of eluents: eluent A, 0 min 95%; 10 min 70%; 25 min 50%; 35 min 30%; and 40 min 95%.

The following 27 standard compounds were used for identification and quantification: malvidin, capsaicin, quercetin, ellagic acid, myricetin, syringic acid, naringenin, sinapic acid, 3-hydroxybenzoic acid, rutin, apigenin, (-)epicatechin, catechin, kaempferol, *p*-coumaric acid, gallic acid, caffeic acid, 4-hydroxybenzoic acid, hippuric acid, protocatechuic acid, salicylic acid, vanillic acid, chlorogenic acid, *t*-cinnamic acid, phloridzin, ferulic acid, and fumaric acid (Sigma-Aldrich, Taufkirchen, Germany). The concentrations of each standard used were 1, 5, 10, 20, 50, and 100 mg/100 mL.

Sensory Quality of Cheese

The sensory quality evaluations of the cheeses were conducted in 3 repetitions (cheeses were produced 3 times) 1 d after their production following the methods described in ISO standards: 4120:2021, 4121:2003, 8587:2006, and 13299:2016 (ISO, 2021a, 2003, 2006, 2016). The analyses were performed by 12 experienced judges, trained in accordance with ISO sensory standards (3972:2011, 4120:2021, 5492:2008, 5495:2005, 8586:2023, 10399:2017, 11132:2021; ISO, 2011, 2021a, 2008b, 2005, 2023, 2017a, 2021b) in individual booths with uniform lighting conditions at the Laboratory for Sensory Analysis (part of the Centre for Innovation and Research on Prohealthy and Safe Food, University of Agriculture in Krakow, Poland), designed in accordance with the ISO 8589:2007 (ISO, 2007) standard. Three types of samples were assessed: I—a control sample without a plant additive, II and III—samples with the addition of dried nettle leaves in the amount of 0.165% and 0.330% (g/100 g of milk), respectively. Results from 3 productions were averaged and means are presented in the figures.

A cheese wheel 8 cm in diameter and 3 cm in height was divided into 4 parts, and each panelist received a quarter of the cheese to evaluate. Samples were served on identical white plates, labeled with random numbers, and arranged in a randomized order. All samples were evaluated 10 min after being taken out of the refrigerator set to 6°C.

The quantitative descriptive analysis (**QDA**) method (ISO 13299:2016; ISO, 2016) was used to assess intensity of sensory attributes. Initially, the panel leader, together with a small group of technologists and experienced sensory assessors, developed a list of potential descriptors. Through open discussion, the most frequently mentioned and relevant descriptors were selected. Definitions were established for each descriptor, and a structured scale from 0 to 5, anchored at both ends, was proposed. The panelists were instructed to evaluate in detail the following sensory attributes of the cheeses: appearance, texture, aroma, and taste (definitions of descriptors are shown in Table 1). The results were presented on column or stellar diagrams; the intensity of attributes was plotted by the distance from the center.

The research activities regarding sensory analysis were approved by the University of Agriculture Ethics Committee (approval no. 189/2024, May 23, 2024), and all participants provided informed consent.

Consumer Testing

Consumer testing was conducted at the Laboratory for Sensory Analysis. The test took place from 1000 to 1300 h. A total of 68 consumers (40 women and 28 men), were recruited among the students and staff of the Faculty of Food Technology, University of Agriculture in Krakow. The age distribution of participants was as follows: 12 aged 18 to 24 yr, 17 aged 25 to 34 yr, 14 aged 35 to 45 yr, 23 aged 46 to 55 yr, and 2 aged over 56 yr.

The cheese samples were served at room temperature on paper plates and were coded with randomly selected 3-digit numbers.

First, the consumers were asked to rate their overall liking of the products using a 5-point hedonic scale, ranging from 5 (“I like very much”) to 1 (“I dislike very much”).

Next, a ranking test (ISO 8587:2006; ISO, 2006) was applied. Consumers were asked to rank the samples in relation to their overall impression and purchase preference, assigning a score of 3 to the most preferred cheese and a score of 1 to the least preferred. In the following stage, the same samples were ranked again, this time considering the consumers’ purchasing preferences with knowledge of the health-promoting properties of cheeses resulting from

Table 1. Definitions of descriptors selected

Attribute	Feature	Scale
General appearance	Shape maintenance: • maintained (typical cheese form, uniform, a flattened cylinder with smooth edges) • slightly changed (a slightly irregular shape, less uniform, with rougher cylinder edges) • changed (a deformed shape, cracked or layered, uneven along the cylinder edges)	☐ maintained ☐ slightly changed ☐ changed
	Surface condition: • maintained (clean, smooth, or clearly imprinted mold pattern on the surface, glossy, with no visible leakage) • slightly changed (clean, but less smooth surface, or with a less distinct mold pattern impression, slightly matte, slightly dried, or with a slight whey leakage) • changed (uneven surface, with numerous impurities, very dry and matte, or with visible whey leakage)	☐ maintained ☐ slightly changed ☐ changed
	Color of surface: • maintained (very typical for fresh rennet cheese, white or cream-colored, uniform across the entire surface with visible pieces of chopped dark green nettle) • slightly changed (a less typical color for fresh rennet cheese, light yellow, less uniform with visible pieces of chopped dark green nettle containing a few brown or dried plant fragments) • changed (an atypical color for rennet cheese, in places yellowish-beige, nonuniform with visible oversized pieces of dark green nettle and numerous brown or dried plant fragments)	☐ maintained ☐ slightly changed ☐ changed
	Appearance in cross-section: • maintained (firm, moist, noncrumbly cheese, uniform, with occasional eyes or fissures; nettle pieces evenly distributed throughout cross-section) • slightly changed (less firm cheese, slightly dry and slightly crumbly, with few eyes or fissures; nettle pieces somewhat unevenly distributed throughout the cross-section) • changed (crumbly cheese, overly dry or soft, with numerous eyes, fissures, and cracks; nettle in large pieces, sparsely distributed across the cross-section)	☐ maintained ☐ slightly changed ☐ changed
Aroma notes	• Cheese (a fresh, slightly milky aroma characteristic of soft rennet cheese) • Sour milk (a fresh and slightly acidic aroma, typical of lactic fermentation) • Nettle leaves or grass (green, herbal aroma, characteristic of nettle or grass) • Rotten (a strong, unpleasant, putrid odor, characteristic of decomposition) • Rancid (a fatty, oxidized, unpleasant odor, characteristic of spoiled or aged fats)	0 1 2 3 4 5 undetectable … strongly detectable
Texture using a fork	• Hardness (the force required to achieve a specified degree of deformation when pressing the sample with a fork)	1 2 3 4 5 very soft … very hard
	• Juiciness (amount of moisture observed as released from the cheese during compression with a fork)	1 2 3 4 5 very dry … very juicy
	• Springiness (how quickly and fully the sample regains its original shape after being compressed with a fork)	1 2 3 4 5 viscous … elastic
	• Brittleness (ease with which the sample breaks or crumbles under pressure)	1 2 3 4 5 compact … crumbly

Continued

Table 1 (Continued). Definitions of descriptors selected

Attribute	Feature	Scale
Texture in the mouth	• Hardness (amount of force felt when biting through the sample)	1 2 3 4 5 very soft … very hard
	• Juiciness (amount of moisture perceived as released from the cheese during compression when biting the sample)	1 2 3 4 5 very dry … very juicy
	• Chewiness (effort perceived as necessary to chew the sample until it is ready for swallowing)	1 2 3 4 5 crumbly … chewy
	• Adhesiveness (the effort perceived as necessary to overcome the stickiness of the sample as it adheres to the mouth surfaces [teeth, tongue, palate])	1 2 3 4 5 non-cohesive … adhesive
Taste notes	• Cheese (a taste characteristic of soft rennet cheese, combining fresh, milky, and fermented dairy notes) • Sour milk (an acidic taste associated with lactic acid in fermented milk or cheese) • Nettle leaves or grass (a vegetal, slightly bitter taste, characteristic of nettle or herbs) • Salty (a basic taste associated with sodium chloride) • Sour (acidic, citrus like, a basic taste associated with lactic and citric acids) • Sweet (a basic taste associated with sucrose) • Bitter (a basic taste associated with caffeine and quinine) • Rotten (an unpleasant taste associated with spoiled or decomposed dairy products) • Rancid (an unpleasant taste associated with oxidized or spoiled fats)	0 1 2 3 4 5 undetectable … strongly detectable
Overall quality		1 very poor, 2 poor, 3 fair, 4 good, 5 very good

the addition of a nettle. Finally, demographic information regarding gender and age was collected.

The research activities regarding consumer assessment were approved by the University of Agriculture Ethics Committee (approval no. 316/2025, September 29, 2025), and all participants provided informed consent.

Statistical Analysis

The results were statistically analyzed using Statistica version 13.3 (TIBCO Software Inc., Boston, MA). Means and standard deviations were calculated. Nonparametric ANOVA (Kruskal–Wallis test) was used regarding chemical analyses: first, to determine the effect of nettle addition, and second, where applicable, to evaluate the influence of storage time. Additionally, Pearson correlation coefficients were calculated, where applicable. The results of the QDA were analyzed to determine the effect of nettle addition using one-way ANOVA and Tukey post hoc test (Baryłko-Pikielna and Matuszewska, 2014). The results of the rank method were analyzed using the Friedman test at a significance level set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Physicochemical Properties of Cheese and Whey

Effects of nettle addition and storage time on physicochemical properties of cheese are presented in Table 2. Nettle addition had a significant ($P < 0.01$) influence on all tested features. It caused a decrease in moisture content, regardless of the level of addition. This phenomenon is common in cheeses containing herbs (Gliguem et al., 2021; Pluta-Kubica et al., 2022, 2024). Nettle addition likely increases solids, reducing moisture and fat contents proportionally. No salt content differences were determined in the cheese samples examined before storage (I: 1.6 ± 0.2, II: 1.6 ± 0.1, and III: 1.5 ± 0.2%, $P = 0.77$). Therefore, the significant decrease in water activity was probably related to the moisture content. Moreover, the samples containing a higher amount of nettle (III) were characterized by a lower fat content than the control (I). However, obtained whey samples were not significantly different in terms of fat content (I: 0.06 ± 0.01, II: 0.05 ± 0.01, and III: 0.05 ± 0.01%, $P = 0.55$). On the other hand, DM amount of the whey obtained during cheese I production seemed lower in comparison to II and III; however, the difference was not significant (I: 6.58 ± 0.18, II: 6.70 ± 0.06, and III: 6.79 ± 0.06, $P = 0.06$). Consequently, the observed reduction in fat content associated with nettle supplementation was not attributable to impaired fat binding during the coagulation process. Lowering of fat content in food products enriched with nettle were previously reported regarding wheat noodles (Ayele et al., 2016) and white bread (Maietti et al., 2021). This may be related to the fact that nettle contains no fat (0.17 g/100 g; Maietti et al., 2021). Higher pH in cheese samples III was determined in comparison to I. However, the differences were somewhat small, with the highest of 0.09 after the first week (Table 2). In general, the pH of the samples was typical for an unripened soft rennet-curd cheese (Pluta-Kubica et al., 2020; Najgebauer-Lejko et al., 2022).

Among the evaluated features, only pH was significantly influenced by storage duration ($P \leq 0.01$). However, only the samples stored for 2 wk were different from the fresh ones. It is possible that LAB caused the observed decrease in pH values by lactic acid fermentation. *Lactococcus* count was still high after the second storage week (Table 2).

Regarding the protein, NaCl, and ash contents in cheese, the effect of nettle addition was examined. Nettle leaf supplementation of cheese affected the protein (I: 14.3 ± 0.6, II: 16.6 ± 0.3, and III: 16.8 ± 0.1%, $P \leq 0.01$) and ash contents (I: 2.4 ± 0.1, II: 2.7 ± 0.1, and III: 3.0 ± 0.2%, $P \leq 0.01$). However, there was no influence on NaCl content (I: 1.6 ± 0.2, II: 1.6 ± 0.1, and III: 1.5 ± 0.2%, $P = 0.77$). The control cheese contained less protein than the samples with nettle. This could have been connected with its higher level of moisture in I than in II and III cheese samples (Table 2). In contrast, only the whey samples obtained during cheese III production contained significantly more protein in comparison to the control whey (I: 0.83 ± 0.03, II: 0.85 ± 0.01, and III: 0.89 ± 0.04%, $P = 0.04$). The ash content gradually increased with increasing nettle addition. Similar influence on ash contents had 5% addition of nettle leaves in bread (Bhusal et al., 2022).

Microbiological Quality of Cheese

Microbial quality, in terms of TVC and *Lactococcus* counts, was not significantly affected by the nettle addition (Table 2). Therefore, it can be concluded that this herb had no influence on the survival of starter cultures used for cheese production nor was it a source of undesirable microbiota. This may be considered as advantage.

A gradual decrease in TVC and *Lactococcus* counts were observed during storage. Similar tendencies were demonstrated in the case of an acid-curd cheese stored for 2 wk (Pluta-Kubica et al., 2021). *Lactococcus* bacteria constitute the majority of the starter culture CHN-19 applied in this study. As is commonly known, the death and lysis of starter cells occur during cheese ripening (McSweeney, 2004). Therefore, these microorganisms gradually lower their number also during cheese storage. Moreover, TVC was significantly ($P < 0.05$) positively correlated with *Lactococcus* counts (r = 0.98).

Table 2. Effects of nettle addition and storage time on physicochemical properties, microbiological quality, and antioxidant activity of cheese

Item	Nettle addition,[1] %	Storage time, wk 0	1	2	3	4	P-value[2] Nettle addition	Storage time
Physicochemical property								
Moisture content, %	0	69.6^{B,a} ± 0.3	68.5^{B,a} ± 0.9	66.3^{B,a} ± 0.8	66.7^{B,a} ± 1.9	65.4^{B,a} ± 1.0		
	0.165	65.1^{A,a} ± 0.8	65.7^{A,a} ± 0.6	65.7^{A,a} ± 1.6	65.5^{A,a} ± 1.0	65.4^{A,a} ± 1.1	<0.01	0.03
	0.330	65.8^{A,a} ± 1.5	65.9^{A,a} ± 0.5	64.6^{A,a} ± 1.1	65.4^{A,a} ± 1.1	64.8^{A,a} ± 1.0		
Fat content, %	0	11.2^{B,a} ± 0.3	11.8^{B,a} ± 0.5	12.0^{B,a} ± 0.3	12.1^{B,a} ± 0.4	12.3^{B,a} ± 0.4		
	0.165	11.4^{B,a} ± 0.5	11.9^{B,a} ± 0.2	11.7^{B,a} ± 0.3	11.2^{B,a} ± 0.8	11.4^{B,a} ± 0.2	<0.01	0.19
	0.330	11.1^{A,a} ± 0.7	11.3^{A,a} ± 0.3	11.1^{A,a} ± 0.2	11.0^{A,a} ± 0.5	10.9^{A,a} ± 0.4		
Water activity	0	0.987^{C,a} ± 0.001	0.987^{C,a} ± 0.001	0.986^{C,a} ± 0.001	0.986^{C,a} ± 0.001	0.985^{C,a} ± 0.001		
	0.165	0.983^{B,a} ± 0.002	0.984^{B,a} ± 0.001	0.984^{B,a} ± 0.002	0.981^{B,a} ± 0.002	0.982^{B,a} ± 0.001	<0.01	0.06
	0.330	0.979^{A,a} ± 0.001	0.982^{A,a} ± 0.002	0.980^{A,a} ± 0.003	0.977^{A,a} ± 0.001	0.977^{A,a} ± 0.001		
pH	0	4.73^{A,b} ± 0.04	4.65^{A,ab} ± 0.07	4.66^{A,a} ± 0.03	4.64^{A,ab} ± 0.09	4.71^{A,ab} ± 0.01		
	0.165	4.70^{A,b} ± 0.03	4.70^{A,ab} ± 0.05	4.68^{A,a} ± 0.02	4.64^{A,ab} ± 0.12	4.72^{A,ab} ± 0.02	<0.01	0.01
	0.330	4.79^{B,b} ± 0.03	4.74^{B,ab} ± 0.07	4.72^{B,a} ± 0.01	4.71^{B,ab} ± 0.09	4.75^{B,ab} ± 0.02		
Microbiological quality[3]								
TVC, log cfu/g	0	9.1^{A,d} ± 0.0	9.0^{A,cd} ± 0.1	8.0^{A,bc} ± 0.4	7.1^{A,ab} ± 0.2	6.9^{A,a} ± 0.4		
	0.165	9.1^{A,d} ± 0.1	8.9^{A,cd} ± 0.1	8.2^{A,bc} ± 0.2	7.5^{A,ab} ± 0.5	6.9^{A,a} ± 0.2	0.93	<0.01
	0.330	9.0^{A,d} ± 0.0	8.8^{A,cd} ± 0.1	8.2^{A,bc} ± 0.1	7.4^{A,ab} ± 0.6	7.0^{A,a} ± 0.3		
Lactococcus count, log cfu/g	0	9.0^{A,d} ± 0.1	8.8^{A,cd} ± 0.2	7.5^{A,bc} ± 0.5	6.6^{A,ab} ± 0.2	6.4^{A,a} ± 0.4		
	0.165	9.1^{A,d} ± 0.0	8.6^{A,cd} ± 0.1	7.7^{A,bc} ± 0.5	6.9^{A,ab} ± 0.6	6.5^{A,a} ± 0.3	0.92	<0.01
	0.330	9.0^{A,d} ± 0.1	8.7^{A,cd} ± 0.2	7.7^{A,bc} ± 0.2	6.6^{A,ab} ± 0.4	6.6^{A,a} ± 0.4		
Yeast and mold count, cfu/g	0	nd[4]	nd	nd	66 ± 92^{a}	158^{Aa} ± 188		
	0.165	nd	nd	nd	nd	nd	0.90	0.37
	0.330	nd	nd	nd	nd	119^{A} ± 186		
Antioxidant activity								
ARP, µmol of TE/g	0	0.09^{A,a} ± 0.03	0.08^{A,a} ± 0.01	0.11^{A,a} ± 0.01	0.10^{A,a} ± 0.01	0.07^{A,a} ± 0.01		
	0.165	0.29^{B,a} ± 0.02	0.29^{B,a} ± 0.06	0.31^{B,a} ± 0.05	0.27^{B,a} ± 0.07	0.23^{B,a} ± 0.03	<0.01	0.51
	0.330	0.43^{C,a} ± 0.04	0.50^{C,a} ± 0.03	0.45^{C,a} ± 0.04	0.43^{C,a} ± 0.11	0.36^{C,a} ± 0.05		
TPC, mg of GAE/100 g	0	31.1^{A,a} ± 7.2	36.7^{A,a} ± 1.6	52.1^{A,b} ± 2.3	49.5^{A,b} ± 5.4	61.0^{A,b} ± 3.6		
	0.165	42.2^{B,a} ± 3.7	48.5^{B,a} ± 4.3	69.0^{B,b} ± 11.5	67.5^{B,b} ± 5.2	79.6^{B,b} ± 6.1	<0.01	<0.01
	0.330	46.7^{B,a} ± 4.3	57.2^{B,a} ± 4.5	67.4^{B,b} ± 8.5	72.6^{B,b} ± 10.6	85.1^{B,b} ± 7.1		

[A–C]Mean values ± SD within a column with different uppercase letters differ ($P \le 0.01$) for nettle addition.
[a–d]Mean values ± SD within a row with different lowercase letters differ ($P \le 0.01$) for storage time.
[1]Nettle addition at the beginning of cheese production (g/100 g of milk).
[2]Probability of treatment effects.
[3]TVC = total viable count; ARP = antiradical power; TE = Trolox equivalent; TPC = total phenolic content; GAE = gallic acid equivalent.
[4]nd = not detected.

Table 3. Effect of nettle addition on polyphenolic profile of cheese and whey

	Cheese				Whey			
Item	0%[1]	0.165%[1]	0.330%[1]	*P*-value[2]	0%[1]	0.165%[1]	0.330%[1]	*P*-value[2]
Hippuric acid, mg/100 g	nd[3]	nd	nd	ne[4]	$15.9^{a} \pm 1.6$	$15.9^{a} \pm 0.9$	$17.4^{a} \pm 2.0$	0.17
Caffeic acid, mg/100 g	nd	$4.6^{a} \pm 0.3$	$9.4^{b} \pm 0.4$	<0.01	nd	$3.8^{a} \pm 0.2$	$8.6^{b} \pm 0.4$	<0.01
p-Coumaric acid, mg/100 g	nd	$0.6^{a} \pm 0.1$	$0.9^{b} \pm 0.1$	<0.01	nd	$0.6^{a} \pm 0.1$	$0.9^{b} \pm 0.0$	<0.01
Rutin, mg/100 g	nd	$3.4^{a} \pm 0.3$	$7.0^{b} \pm 0.3$	<0.01	nd	$0.9^{a} \pm 0.3$	$1.9^{b} \pm 0.2$	<0.01
Ferulic acid, mg/100 g	$20.7^{a} \pm 0.9$	$22.4^{b} \pm 0.8$	$21.8^{b} \pm 0.9$	<0.01	nd	$0.4^{a} \pm 0.3$	$0.7^{a} \pm 0.1$	0.09
Compound A, area/100 g	$2.3^{a} \pm 1.0$	$2.2^{a} \pm 0.5$	$2.1^{a} \pm 0.8$	0.75	$14.1^{a} \pm 1.9$	$13.9^{a} \pm 1.8$	$16.4^{a} \pm 1.2$	0.01
Compound B, area/100 g	$2.4^{a} \pm 0.7$	$2.6^{a} \pm 0.5$	$3.0^{a} \pm 1.9$	0.79	$13.8^{a} \pm 9.9$	$9.2^{a} \pm 5.3$	$12.8^{a} \pm 4.6$	0.43
Compound C, area/100 g	$4.6^{a} \pm 0.3$	$5.3^{b} \pm 0.4$	$5.3^{b} \pm 0.2$	<0.01	$0.5^{a} \pm 0.0$	$0.6^{ab} \pm 0.1$	$0.7^{b} \pm 0.1$	<0.01
Compound D, area/100 g	nd	$1.7^{a} \pm 0.1$	$3.6^{b} \pm 0.1$	<0.01	nd	$2.3^{a} \pm 0.1$	$5.1^{b} \pm 0.2$	<0.01

[a,b]Mean values ± SD within a row with different superscript letters differ ($P \leq 0.01$) for nettle addition separately for cheese and whey samples.

[1]Nettle addition at the beginning of cheese production (g/100 g of milk).

[2]Probability of treatment effect—nettle addition.

[3]nd = not detected.

[4]ne = not examined.

Yeast and mold were not detected in any samples from the beginning to the second week of storage. At the third week, these microorganisms were found only in the control. They were also present in samples I and III at the end of storage. It can be concluded that the addition of nettle slowed down the yeast and mold growth in cheese. This may indicate that the addition of dried nettle leaves prolongs the shelf-life of unripened soft cheese. A similar effect was demonstrated for *Allium roseum* in double cream cheese (Gliguem et al., 2021).

Antioxidant Activity and Total Content of Polyphenols in Cheese and Whey

A gradual significant ($P < 0.01$) increase in antioxidant activity of cheese due to an amount of added nettle was observed (Table 2). The samples enriched with nettle exhibited also a higher total phenolic content in comparison to the control. Since nettle leaves are a rich source of polyphenols and antioxidants (Repajić et al., 2021), this effect was expected. The results also indicate that a sufficiently high dose of the additive was applied in this research. Moreover, ARP was significantly ($P < 0.05$) positively correlated with TPC (r = 0.49).

Although time of storage did not affect the antioxidant activity, the amount of GAE significantly increased during storage ($P \leq 0.01$). However, the Folin–Ciocalteu reagent can react with many nonphenolic compounds, including some AA (Torres et al., 2024). Previous results have shown that the addition of herbs to cheese causes an intensification of proteolysis (Coşkun and Tunçtürk, 2000; Tarakci and Temiz, 2009; Najgebauer-Lejko et al., 2022). Probably the reaction with free AA could have caused the observed increase of TPC during storage. Proteolytic changes will be the subject of the next stage of research on cheeses with nettle.

Increasing the nettle addition also affected both ARP ($P \leq 0.01$) and TPC ($P \leq 0.01$) values of the whey samples obtained after cheese production. Antioxidant activity was 0.02 ± 0.00, 0.28 ± 0.01, and 0.50 ± 0.03 µmol of TE/g, whereas TPC equaled 12.33 ± 0.69, 15.62 ± 1.57, and 18.63 ± 0.95 GAE/100 g, respectively. A significant ($P < 0.01$) difference was found when comparing the control sample and the one containing the highest proportion of nettle, for both features. Whey consists mainly of water that is known to be a good solvent for phenolic acids and glycosides (Flórez et al., 2022). These antioxidants may be present in nettle, and therefore, their occurrence could have caused these results. Moreover, similarly to the cheese analysis, ARP of whey was significantly ($P < 0.05$) positively correlated with TPC of whey (r = 0.91). Therefore, the conducted studies have shown a beneficial effect of dried nettle leaves available in the market, manifested by an increase in the oxidation-reduction potential both in model unripened cheeses and in the whey remaining after their production. Moreover, the tested whey can potentially become a raw material for the development of whey-based functional products with antioxidant properties.

Polyphenolic Profile in Nettle Leaves, Whey, and Cheese

Five out of 27 polyphenols were identified in the examined samples: hippuric acid, caffeic acid, *p*-coumaric acid, rutin, and ferulic acid (Table 3). Four of these compounds were determined in the nettle leaves: caffeic acid, *p*-coumaric acid, rutin, and ferulic acid. These substances were present in the following amounts: 3,665.9 ± 404.5, 246 ± 35.3, 1,232.5 ± 87.3, and 310.7 ± 23.7 mg/100 g, respectively. Hippuric acid was detected only in the whey samples (Table 3). This compound, absorbed from

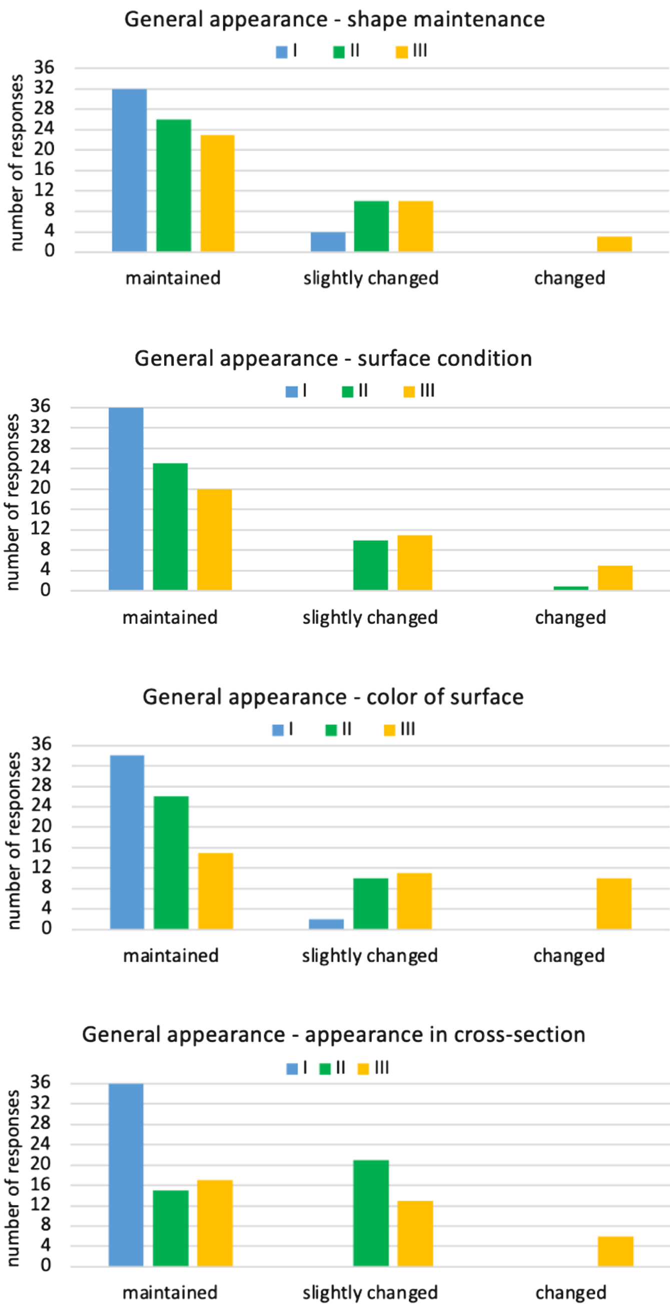

Figure 1. Sensory analysis of the general appearance of cheeses. I, II, and III refer to 0%, 0.165%, and 0.330%, respectively, of nettle addition at the beginning of cheese production (g/100 g of milk).

feed, is naturally present in cow and goat milk, and is soluble in water (Carpio et al., 2013). Therefore, it most likely was completely dissolved in the whey samples and its presence was not connected with the nettle addition. Rutin, and caffeic and *p*-coumaric acids originated from the plant additive. Therefore, they were detected in the cheeses II and III and the whey samples derived from them. The amounts of these polyphenols were dependent on the level of nettle addition. These compounds possess antioxidant and antimicrobial properties (Stojković et al., 2013). It is possible that rutin, and caffeic and *p*-coumaric acids slowed down the yeast and mold growth in cheese II and III samples and were the reason for their elevated ARP and TPC values (Table 2). Ferulic acid, like hippuric acid, originates from feed and is a natural component of milk (Soberon et al., 2012). It was detected in all cheese and almost all whey samples, except the control one. Moreover, ferulic acid was present in the nettle leaves, which increased its quantity in cheese II and III samples. Ferulic acid is an anticarcinogenic and antioxidant compound (Soberon et al., 2012).

Additionally, the presence of 4 unidentified compounds was revealed. All of them were detected in the nettle leaves: A—52.2 ± 24.5, B—32.4 ± 7.1, C—112.9 ± 36.6, and D—553.3 ± 97.4 peak area/100 g. The compounds A, B, and C were also found in all cheese and whey samples, whereas D was detected in the cheeses II and III and the whey samples derived from them. Therefore, the polyphenols A, B, and C, in contrast to D, were probably the compounds originating from fodder. The presence of the compound D, similarly to rutin, and caffeic and *p*-coumaric acids, was dependent from the level of nettle addition and could have contributed to the increased antioxidant activity of cheese II and III samples and their corresponding whey samples.

Sensory Quality of Cheese

Cheese control and with added nettle leaves samples were evaluated for sensory attributes using a QDA. The data are presented in Figure 1 and Table 4.

Initially, the panelists visually assessed the appearance of the cheese samples (Figure 1). The control sample was rated very highly in terms of shape, surface condition, color, and cross-sectional appearance. However, with the addition of dried nettle leaves at concentrations of 0.165% and 0.330% (g/100 g of milk), the aforementioned aspects of overall appearance of the samples were rated progressively lower compared with the control. Approximately half of the responses (out of 36) indicated that the cross-sectional appearance of cheese II was "slightly changed," whereas the sample containing the highest nettle concentration was often described as "slightly changed" and "changed." Thus, the addition of dried nettle leaves resulted in a decline in the visual quality of the cheese samples, particularly in the sample with the 0.330% nettle content.

The results indicate that some sensory attributes presented in Table 4 were influenced by the addition and concentration of nettle leaves. The texture assessed during pressing the cheese with a fork, as well as the texture evaluated in the mouth during chewing, appeared to be similar across all samples (Table 4). However, the

Table 4. Sensory analysis of the texture, aroma, and taste of cheeses

Item	Cheese			P-value[2]
	0%[1]	0.165%[1]	0.330%[1]	
Texture using fork				
Hardness	$3.33^{a} \pm 0.63$	$3.69^{ab} \pm 0.67$	$3.83^{b} \pm 0.70$	<0.01
Juiciness	$2.86^{a} \pm 0.64$	$2.64^{a} \pm 1.02$	$2.47^{a} \pm 0.94$	0.18
Springiness	$3.19^{a} \pm 0.79$	$3.08^{a} \pm 0.77$	$3.14^{a} \pm 0.72$	0.83
Brittleness	$2.83^{a} \pm 0.56$	$2.89^{a} \pm 0.89$	$2.81^{a} \pm 1.04$	0.91
Texture in the mouth				
Hardness	$2.86^{a} \pm 0.72$	$3.06^{a} \pm 0.68$	$3.17^{a} \pm 0.61$	0.17
Juiciness	$3.39^{b} \pm 0.60$	$2.97^{a} \pm 0.82$	$2.75^{a} \pm 0.84$	<0.01
Chewiness	$2.64^{a} \pm 0.72$	$2.86^{a} \pm 0.65$	$2.67^{a} \pm 0.63$	0.42
Adhesiveness	$2.42^{a} \pm 0.84$	$2.54^{a} \pm 0.92$	$2.83^{a} \pm 0.94$	0.14
Aroma notes				
Cheese	$3.97^{b} \pm 0.97$	$3.19^{a} \pm 1.06$	$2.92^{a} \pm 1.20$	<0.01
Sour milk	$3.06^{b} \pm 1.35$	$2.42^{ab} \pm 1.34$	$1.92^{a} \pm 1.00$	<0.01
Nettle leaves	$0.00^{a} \pm 0.00$	$1.86^{b} \pm 1.33$	$3.08^{c} \pm 1.52$	<0.01
Rotten	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.11^{b} \pm 0.32$	<0.05
Rancid	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.06^{a} \pm 0.23$	0.13
Taste notes				
Cheese	$4.08^{b} \pm 0.97$	$3.42^{a} \pm 1.11$	$2.92^{a} \pm 1.16$	<0.01
Sour milk	$2.61^{b} \pm 1.27$	$3.11^{b} \pm 1.19$	$1.78^{a} \pm 1.33$	<0.01
Nettle leaves	$0.00^{a} \pm 0.00$	$3.36^{b} \pm 1.22$	$4.08^{c} \pm 1.02$	<0.01
Salty	$3.64^{a} \pm 1.13$	$3.14^{a} \pm 1.25$	$3.31^{a} \pm 1.01$	0.17
Sour	$2.11^{a} \pm 1.06$	$2.19^{a} \pm 1.06$	$2.33^{a} \pm 1.12$	0.68
Sweet	$0.72^{a} \pm 0.88$	$0.67^{a} \pm 0.93$	$0.50^{a} \pm 0.77$	0.53
Bitter	$1.03^{a} \pm 1.44$	$1.53^{ab} \pm 1.52$	$2.06^{b} \pm 1.88$	<0.05
Rotten	$0.11^{a} \pm 0.67$	$0.03^{a} \pm 0.17$	$0.08^{a} \pm 0.37$	0.73
Rancid	$0.00^{a} \pm 0.00$	$0.06^{a} \pm 0.23$	$0.08^{a} \pm 0.28$	0.24
Overall quality	$4.56^{b} \pm 0.73$	$3.42^{a} \pm 0.97$	$2.89^{a} \pm 1.17$	<0.01

[a–c]Mean values ± SD within a row with different superscript letters differ ($P \leq 0.05$) for nettle addition.

[1]Nettle addition at the beginning of cheese production (g/100 g of milk).

[2]Probability of treatment effect—nettle addition.

addition of nettle significantly ($P \leq 0.01$) increased the hardness of cheese during pressing with a fork while reducing the juiciness of the cheese compared with the control sample without nettle (sample I).

The incorporation of nettle leaves resulted in a significant ($P < 0.01$) decrease in the characteristic aroma and taste notes of cheese and sour milk, which are typical for these types of dairy products. Furthermore, the intensity of the nettle leaves aroma and taste was approximately one point higher in the cheese sample with the higher concentration of leaves. The addition of nettle also significantly ($P \leq 0.01$) increased the intensity of bitterness in the cheese. The overall quality of the samples gradually declined with increasing nettle concentration, with scores decreasing from 4.56 (sample I), to 3.42 (sample II), and 2.89 (sample III). A similar observation was reported by Awda et al. (2019), who found that the incorporation of a high concentration of celery leaves in soft cheese increased bitterness and decreased overall acceptability of the product. Overall, the sensory evaluation conducted by the panelists (Figure 1 and Table 4) demonstrated that the cheese without nettle had superior sensory characteristics compared with the nettle-enriched variants. These findings are consistent with the results of Setyawardani et al. (2023), who also reported poorer sensory quality in cottage cheese after the addition of various herbal extracts. The authors observed that higher concentration of herb extracts increased bitterness and grassy aroma. In contrast, Algarni (2016) found that the addition of thyme, cumin, and turmeric extracts to soft cheese did not significantly alter its organoleptic properties compared with the control. However, during a 30-d storage period, the addition of these extracts was shown to enhance the sensory evaluation of fresh soft cheese, with total exceeding those of the fresh samples.

Consumer Testing

The control cheese received very high positive scores for overall acceptability, with 84% of responses indicating "I like it" or "I like it very much" (Figure 2). The addition of 0.165% dried nettle leaves reduced the proportion of positive responses to 70%, whereas the addition of 0.330% further decreased acceptability to 48.5% of the positive responses.

The ranking test (Table 5) indicated a lower purchase intention for cheese samples with added nettle leaves. Initially, the consumers ranked the samples based on

Table 5. The results of the ranking test[1]

Ranking test	Cheese		
	0%[2]	0.165%[2]	0.330%[2]
Ranking scores related to overall impression and purchase preference, without prior knowledge of the pro-health properties of the cheese with nettle	160[a]	139[b]	109[c]
Ranking scores related to overall impression and purchase preference after assessors were informed about the pro-health properties of the cheese enriched with nettle	127[b]	156[a]	125[b]

[a–c]Ranking scores within a row with different superscript letters differ ($P \leq 0.05$).

[1]The values in the table correspond to the total sum of ranks assigned by the panelists, where 3 points was given to the most preferred sample, 2 points to the moderately preferred one, and 1 point to the least preferred.

[2]Nettle addition at the beginning of cheese production (g/100 g of milk).

overall impression and purchase intention as follows: (1) control sample without nettle, most preferred; (2) cheese with a lower concentration of nettle (0.165% g/100 g of milk), intermediate preference; and (3) cheese with a higher concentration of nettle (0.330% g/100 g of milk), least preferred ($P \leq 0.05$). The preference order corresponded to the overall quality of the products assessed by the trained panelists (Table 4). However, after the assessors were informed about the potential health benefits of nettle-enriched cheese, their attitudes shifted (Table 5). The purchase intentions for the control sample and the sample with the 0.330% nettle concentration were comparable, yet both were lower than those for the cheese containing a 0.165% of nettle, which was significantly the most preferred sample ($P \leq 0.05$). The fact that cheese with a lower amount of nettle was more preferred than the control sample without nettle indicates satisfactory product quality and suggests its potential for commercial success as an alternative to other soft cheeses with herbal additives. It is worth noting that the products were evaluated by individuals affiliated with the Faculty of Food Technology. Their nutritional awareness and food knowledge might have influenced their evaluation of the cheeses. However, the participants changed their preferences only after being informed about the health-promoting properties of the nettle-enriched cheeses. This suggests that less nutrition-conscious consumers could also be inclined to purchase such cheese after appropriate advertising and education, despite its slightly lower sensory attributes, such as less intense cheese and sour notes or lower juiciness compared with the cheese without additives.

CONCLUSIONS

The research results presented in this study clearly contributed to expanding knowledge on the effect of dried nettle leaves on microflora and antioxidant activity in cheeses. This additive slowed down the growth of yeast and molds, increased the antioxidant activity and elevated the contents

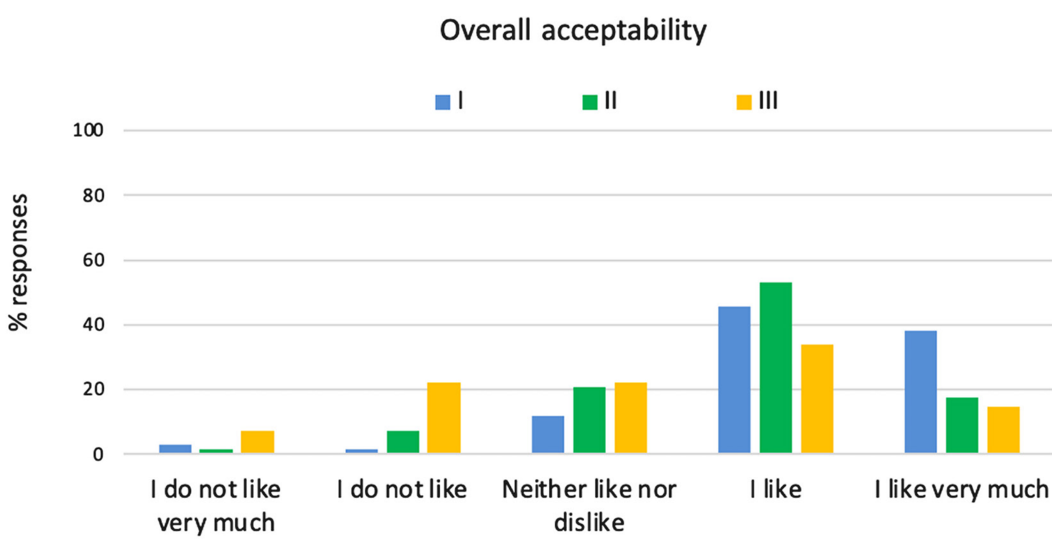

Figure 2. Sensory analysis of the overall acceptability of cheeses. I, II, and III refer to 0%, 0.165%, and 0.330%, respectively, of nettle addition at the beginning of cheese production (g/100 g of milk).

of total polyphenols, caffeic acid, *p*-coumaric acid, rutin, and ferulic acid. In general, based on all sensory evaluation and consumer testing results, the addition of a lower amount (0.165%) of dried nettle leaves appeared to be the most favorable option, balancing improved nutritional and antioxidant value with acceptable sensory characteristics and acceptability. Additionally, the study allowed determining the antioxidant properties of the obtained whey, which will enable planning its use in further studies aiming to create a fermented whey beverage. Further research will aim to examine other cheese characteristics connected with its shelf-life, such as oxidative stability of fat, fatty acid profile and proteolysis during storage.

NOTES

The analyses of the physicochemical properties of cheese and whey, microbiological quality of cheese, as well as antioxidant activity and total content of polyphenols in cheese and whey were financed by the National Science Centre, Poland–MINIATURA 7, grant no. 2023/07/X/NZ9/00618. The analyses of the polyphenolic profile in nettle leaves, whey, and cheese as well as the sensory quality of cheese were supported by funds from the project: the task titled "Research network of life science universities for the development of the Polish dairy sector—Research project" financed under the targeted subsidy of the Minister of Science and Higher Education (Warsaw, Poland; no. MEiN/2023/DPI/2862). For publication, the funds from the project "Research network of life science universities for the development of the Polish dairy sector—Research project" financed under the targeted subsidy of the Minister of Science and Higher Education (Warsaw, Poland; no. MEiN/2023/DPI/2862) were used. The research activities regarding sensory analysis were approved by the University of Agriculture Ethics Committee (approval no. 189/2024, May 23, 2024, and approval no. 316/2025, September 29, 2025), and all participants provided informed consent. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: ARP = antiradical power; DPPH = 2,2-diphenyl-1-picrylhydrazyl; EC_{50} = amount of sample required to decrease the DPPH radical concentration by 50%; GAE = gallic acid equivalent; LAB = lactic acid bacteria; QDA = quantitative descriptive analysis; TE = Trolox equivalent; TPC = total phenolic content; TVC = total viable count.

REFERENCES

Algarni, E. H. A. 2016. Soft cheese supplemented with thyme, cumin and turmeric herbs to increase shelf life during storage period. Adv. Environ. Biol. 10:227–236.

AOAC International. 2007. Chapter 33. Dairy Products. Official Methods of Analysis of AOAC International. 18th ed. AOAC International.

Awda, J. M., H. A. Awad, M. A. Alssirag, and D. A. Alfalahi. 2019. Extend the shelf life and improving sensory properties of white soft cheese by adding celery . . . leaves. Iraqi J. Agric. Sci. 50:1661–1667. https://doi.org/10.36103/ijas.v50i6.857.

Aydın, E., and Z. Tarakçı. 2021. Effects of different types of herbs on colour and texture properties of Kashar cheese. Food Health 7:120–127. https://doi.org/10.3153/FH21013.

Ayele, D., G. Desse Haki, K. Abegaz Ali, B. Berhanu, D. Alemayehu, G. Desse, K. Abegaz, B. Berhanu Desalegn, and D. Getahun. 2016. Proximate, mineral composition and sensory acceptability of home made noodles from stinging nettle (*Urtica simensis*) leaves and wheat flour blends. Int. J. Food Sci. Nutr. Eng. 6:55–61.

Baryłko-Pikielna, N., and I. Matuszewska. 2014. Rozdział 10. Metody sensorycznej analizy opisowej. Wydawnictwo Naukowe PTTŻ, Kraków.

Bhusal, K. K., S. K. Magar, R. Thapa, A. Lamsal, S. Bhandari, R. Maharjan, S. Shrestha, and J. Shrestha. 2022. Nutritional and pharmacological importance of stinging nettle (*Urtica dioica* L.): A review. Heliyon 8:e09717. https://doi.org/10.1016/j.heliyon.2022.e09717.

Carpio, A., D. Bonilla-Valverde, C. Arce, V. Rodríguez-Estévez, M. Sánchez-Rodríguez, L. Arce, and M. Valcárcel. 2013. Evaluation of hippuric acid content in goat milk as a marker of feeding regimen. J. Dairy Sci. 96:5426–5434. https://doi.org/10.3168/jds.2012-6396.

Codex Alimentarius Commission. 2010. Codex group standard for unripened cheese including fresh cheese. CODEX STAN 221-2001. FAO/WHO Codex Alimentarius Commission, Rome, Italy.

Coşkun, H., and Y. Tunçtürk. 2000. The effect of *Allium* sp. on the extension of lipolysis and proteolysis in Van herby cheese during maturation. Nahrung - Food 44:52–55. https://doi.org/10.1002/(SICI)1521-3803(20000101)44:1<52::AID-FOOD52>3.0.CO;2-B.

Fiol, C., D. Prado, M. Mora, and J. I. Alava. 2016. Nettle cheese: Using nettle leaves (*Urtica dioica*) to coagulate milk in the fresh cheese making process. Int. J. Gastron. Food Sci. 4:19–24. https://doi.org/10.1016/j.ijgfs.2016.05.001.

Flórez, M., P. Cazón, and M. Vázquez. 2022. Antioxidant extracts of nettle (*Urtica dioica*) leaves: Evaluation of extraction techniques and solvents. Molecules 27:6015. https://doi.org/10.3390/molecules27186015.

Gliguem, H., D. Ben Hassine, L. Ben Haj Said, I. Ben Tekaya, R. Rahmani, and S. Bellagha. 2021. Supplementation of double cream cheese with *Allium roseum*: Effects on quality improvement and shelf-life extension. Foods 10:1276. https://doi.org/10.3390/foods10061276.

Hamedi, A., M. Bayat, Y. Asemani, and Z. Amirghofran. 2022. A review of potential anti-cancer properties of some selected medicinal plants grown in Iran. J. Herb. Med. 33:100557. https://doi.org/10.1016/j.hermed.2022.100557.

Ioannidou, M. D., M. Maggira, and G. Samouris. 2022. Physicochemical characteristics, fatty acids profile and lipid oxidation during ripening of graviera cheese produced with raw and pasteurized milk. Foods 11:2138. https://doi.org/10.3390/foods11142138.

ISO. 2003. ISO 4121:2003. Sensory analysis—Guidelines for the use of quantitative response scales. International Organization for Standardization (ISO), Geneva, Switzerland.

ISO. 2004. ISO 5534:2004. Cheese and processed cheese—Determination of the total solids content. International Organization for Standardization (ISO), Geneva, Switzerland.

ISO. 2005. ISO 5495:2005. Sensory analysis—Methodology—Paired comparison test. International Organization for Standardization (ISO), Geneva, Switzerland.

ISO. 2006. ISO 8587:2006. Sensory analysis—Methodology—Ranking. International Organization for Standardization (ISO), Geneva, Switzerland.

ISO. 2007. ISO 8589:2007. Sensory analysis—General guidance for the design of test rooms. International Organization for Standardization (ISO), Geneva, Switzerland.

ISO. 2008a. ISO 3433:2008. Cheese—Determination of fat content—Van Gulik method. International Organization for Standardization (ISO), Geneva, Switzerland.

ISO. 2008b. ISO 5492:2008. Sensory analysis—Vocabulary. International Organization for Standardization (ISO), Geneva, Switzerland.
ISO. 2011. ISO 3972:2011. Sensory analysis—Methodology—Method of investigating sensitivity of taste. International Organization for Standardization (ISO), Geneva, Switzerland.
ISO. 2016. ISO 13299:2016. Sensory analysis—Methodology—General guidance for establishing a sensory profile. International Organization for Standardization (ISO), Geneva, Switzerland.
ISO. 2017a. ISO 10399:2017. Sensory analysis—Methodology—Duo-trio test. International Organization for Standardization (ISO), Geneva, Switzerland.
ISO. 2017b. ISO 18787:2017. Foodstuffs—Determination of water activity. International Organization for Standardization (ISO), Geneva, Switzerland.
ISO. 2021a. ISO 4120:2021. Methodology—Triangle test. International Organization for Standardization (ISO), Geneva, Switzerland.
ISO. 2021b. ISO 11132:2021. Sensory analysis—Methodology—Guidelines for the measurement of the performance of a quantitative descriptive sensory panel. International Organization for Standardization (ISO), Geneva, Switzerland.
ISO. 2023. ISO 8586:2023. Sensory analysis—Selection and training of sensory assessors. International Organization for Standardization (ISO), Geneva, Switzerland.
Klimczak, I., M. Małecka, M. Szlachta, and A. Gliszczyńska-Świgło. 2007. Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. J. Food Compos. Anal. 20:313–322. https://doi.org/10.1016/j.jfca.2006.02.012.
Maietti, A., P. Tedeschi, M. Catani, C. Stevanin, L. Pasti, A. Cavazzini, and N. Marchetti. 2021. Nutrient composition and antioxidant performances of bread-making products enriched with stinging nettle (*Urtica dioica*) leaves. Foods 10:938. https://doi.org/10.3390/foods10050938.
McSweeney, P. L. H. 2004. Biochemistry of cheese ripening. Int. J. Dairy Technol. 57:127–144. https://doi.org/10.1111/j.1471-0307.2004.00147.x.
Najgebauer-Lejko, D., A. Pluta-Kubica, J. Domagała, K. Turek, I. Duda, and J. Golian. 2022. Effect of bear garlic addition on the chemical composition, microbiological quality, antioxidant capacity, and degree of proteolysis in soft rennet cheeses produced from milk of Polish Red and Polish Holstein-Friesian cows. Molecules 27:8930. https://doi.org/10.3390/molecules27248930.
Ong, L., and N. P. Shah. 2009. Probiotic Cheddar cheese: Influence of ripening temperatures on survival of probiotic microorganisms, cheese composition and organic acid profiles. Lebensm. Wiss. Technol. 42:1260–1268. https://doi.org/10.1016/j.lwt.2009.01.011.
Pluta-Kubica, A., E. Jamróz, L. Juszczak, P. Krzyściak, and M. Zimowska. 2021. Characterization of furcellaran-whey protein isolate films with green tea or pu-erh extracts and their application as packaging of an acid-curd cheese. Food Bioprocess Technol. 14:78–92. https://doi.org/10.1007/s11947-020-02570-2.
Pluta-Kubica, A., E. Jamróz, A. Kawecka, L. Juszczak, and P. Krzyściak. 2020. Active edible furcellaran/whey protein films with yerba mate and white tea extracts: Preparation, characterization and its application to fresh soft rennet-curd cheese. Int. J. Biol. Macromol. 155:1307–1316. https://doi.org/10.1016/j.ijbiomac.2019.11.102.
Pluta-Kubica, A., D. Najgebauer-Lejko, J. Domagała, J. Lakatošová, M. Šnirc, and J. Golian. 2024. Sensory quality, volatile compounds, and physical properties of sheep's milk cheese with herbs (*Allium ursinum* L.). Molecules 29:5999. https://doi.org/10.3390/molecules29245999.
Pluta-Kubica, A., D. Najgebauer-Lejko, J. Domagała, J. Štefániková, and J. Golian. 2022. The effect of cow breed and wild garlic leaves (*Allium ursinum* L.) on the sensory quality, volatile compounds, and physical properties of unripened soft rennet-curd cheese. Foods 11:3948. https://doi.org/10.3390/foods11243948.
PN-EN ISO 4833-1:2013-12. 2013. Microbiology of the food chain—Horizontal method for the enumeration of microorganisms—Part 1: Colony count at 30 degrees C by the pour plate technique. International Organization for Standardization (ISO), Geneva, Switzerland.
PN-ISO 6611:2007. 2007. Milk and milk products—Enumeration of colony-forming units of yeasts and/or moulds—Colony-count technique at 25 degrees C. International Organization for Standardization (ISO), Geneva, Switzerland.
PN-EN ISO 6887-5:2010. 2010. Microbiology of food and animal feeding stuffs—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—Part 5: Specific rules for the preparation of milk and milk products. International Organization for Standardization (ISO), Geneva, Switzerland.
PN-ISO 9622:2015-09. 2015. Milk and liquid dairy products—Guidelines for the use of mid-infrared spectrometry. International Organization for Standardization (ISO), Geneva, Switzerland.
Repajić, M., E. Cegledi, Z. Zorić, S. Pedisić, I. Elez Garofulić, S. Radman, I. Palčić, and V. Dragović-Uzelac. 2021. Bioactive compounds in wild nettle (*Urtica dioica* L.) leaves and stalks: Polyphenols and pigments upon seasonal and habitat variations. Foods 10:190. https://doi.org/10.3390/foods10010190.
Setyawardani, T., J. Sumarmono, H. Dwiyanti, and N. D. Arkan. 2023. Yield, composition, texture, and sensory characteristics of cottage cheese produced with the incorporation of different herb extracts. Anim. Prod. (Purwokerto) 25:109–117. https://doi.org/10.20884/1.jap.2023.25.2.197.
Soberon, M. A., J. H. Cherney, R. H. Liu, D. A. Ross, and D. J. R. Cherney. 2012. Free ferulic acid uptake in lactating cows. J. Dairy Sci. 95:6563–6570. https://doi.org/10.3168/jds.2011-5018.
Stojković, D., J. Petrović, M. Soković, J. Glamočlija, J. Kukić-Marković, and S. Petrović. 2013. In situ antioxidant and antimicrobial activities of naturally occurring caffeic acid, p-coumaric acid and rutin, using food systems. J. Sci. Food Agric. 93:3205–3208. https://doi.org/10.1002/jsfa.6156.
Tarakci, Z., and H. Temiz. 2009. A review of the chemical, biochemical and antimicrobial aspects of Turkish Otlu (herby) cheese. Int. J. Dairy Technol. 62:354–360. https://doi.org/10.1111/j.1471-0307.2009.00495.x.
Torres, P., S. Osaki, E. Silveira, D. Y. A. C. dos Santos, and F. Chow. 2024. Comprehensive evaluation of Folin-Ciocalteu assay for total phenolic quantification in algae (Chlorophyta, Phaeophyceae, and Rhodophyta). Algal Res. 80:103503. https://doi.org/10.1016/j.algal.2024.103503.
Upton, R. 2013. Stinging nettles leaf (*Urtica dioica* L.): Extraordinary vegetable medicine. J. Herb. Med. 3:9–38. https://doi.org/10.1016/j.hermed.2012.11.001.